REMARKS

Applicants have cancelled Claims 5, 6, 7, 15, 16, 32, 36, 37, 38, 46, and 47. Applicants reserve the right to prosecute any canceled subject matter in a continuation application.

Applicants have amended the claims to cover particularly preferred embodiments of Applicants' invention for reducing loss of affinity particles used in one or more steps of a method for isolating proteinaceous molecules. In particular, the claims of this application are directed to a method for isolating a fusion protein comprising a peptide, polypeptide, or protein fused with "an affinity peptide tag" consisting of a plurality of consecutive histidine residues using metal-chelate affinity particles in the presence of a detergent in the range of 0.0005% - 2% (v/v) sufficient to reduce loss of the metal-chelate affinity particles during any separation or collection step in which the affinity beads are collected and/or separated from other components in a sample (see, Claims 2, 34, 64, and 66, and claims depending therefrom). In a preferred embodiment, the metal-chelate affinity particles are magnetic (see, Claims 64 and 66, and claims depending therefrom). Furthermore, in a particularly preferred embodiment, the invention provides a method for reducing loss of metal-chelate affinity particles wherein the metal-chelate affinity particles are nickel-nitrilotriacetic acid (Ni-NTA) agarose beads (see, new Claim 70). Support for the amendments to the claims to cover the various preferred embodiments of this invention for reducing metal-chelate affinity particle loss using a detergent in a method for isolating a fusion protein is found in the specification. See, in the specification, e.g., p. 8, lines 20-22 (use of Ni-NTA particles); p. 9, lines 1-30 (use of affinity particles, use of magnetic affinity particles, use of fusion protein comprising consecutive histidine residues as an affinity tag); p. 10, lines 1-13 (use of detergent in one or more steps of methods); p. 11, lines 1-14 (use of detergent with affinity particles and magnetic affinity particles, preferred concentration range of detergent); Examples 1-6 at p. 20, line 1-p. 28, line 24 (methods employing various concentrations of various detergents to reduce loss of affinity particles and to increase yields, including use of histidine-tagged fusion proteins and Ni-NTA magnetic affinity particles). Accordingly, the amendments add no new matter.

Claim 3, which depends from independent Claim 2, has been amended to replace the term "separation" with the term "collecting", which has antecedent support in Claim 2. This particular embodiment of the invention is described in the specification (see, e.g., p. 10, lines 12-13 of the specification). Accordingly, the amendment adds no new matter, but maintains consistent use of terms throughout the claims.

Applicants have amended Claims 8, 39, and 48, to adjust dependencies from canceled claims to an appropriate claim carried forward in this application. Accordingly, the amendments add no new matter.

Applicants have also amended Claims 13, 14, 19, 44, and 45 to incorporate terms used in the particular claims from which they depend. Accordingly, the amendments are made to maintain consistent use of terms throughout the claims and, thus, add no new matter.

Entry of the amendments is respectfully requested.

In Paper No. 7, the Examiner maintained rejections of certain claims under 35 USC § 102 as anticipated by Zhang or Weisburg. The Examiner also maintained his rejections of certain claims under 35 USC § 103 as obvious over Weisburg and also over the combinations of Zhang and McCoy, Zhang and Gallant, Zhang and Stein, Zhang and Tsaur, and Zhang and Taoda.

Applicants have previously reviewed and characterized the various references and combinations of references relied on the by the Examiner (see, Applicants' prior Response, filed November 19, 2001). Applicants note that none of the references either alone or in the combinations cited in the Office Action teach or suggest using detergent to reduce metal-chelate affinity particle loss in a method for isolating a fusion protein comprising a peptide, polypeptide, or protein and "an affinity peptide tag" consisting of a plurality of consecutive histidine residues, such as a 6xHis tag. Furthermore, in the case of rejections under 35 USC § 103, Applicants find no evidence as to why persons skilled in this art would even be motivated to combine the references as envisioned by the Examiner to arrive at the invention now claimed in this application. Applicants respectfully traverse the current rejections for the reasons already of record and for the additional reasons indicated below.

At the outset, Applicants note that their invention is a *method* that employs a particular range of detergent to reduce loss of metal-chelate affinity particles in a method for isolating affinity tagged fusion proteins. The inventive feature of Applicants' invention does *not* reside in one or a combination of elements of any *composition* recited in the claims. Examples of compositions, e.g., a fusion protein comprising an affinity peptide tag consisting of consecutive histidine residues (e.g., 6xHis tag) and metal-chelate affinity particles, such as Ni-NTA beads, that may be employed in the claimed invention are known to persons skilled in this art. Applicants have discovered an improvement of prior art affinity particle-based methods by solving a practical problem of *how* to reduce loss of metal-chelate affinity particles during manipulations that are routinely carried out in affinity particle separation methods. Loss of significant amounts of metal-chelate affinity particles during standard manipulations of the particles (e.g., collection, separation, washing) is a significant and undesirable phenomenon that Applicants have observed, documented, and solved by contacting the affinity particles with detergent according to the invention. See, e.g., in Example 1, p. 20, line 27-p. 21, line 2 and Table 1 on p. 21 of the specification; in Example 2, p. 22, lines 15-20 and Table 2 on p. 22 of the specification; in Example 3, p. 23, lines 13-19

and Table 3 on p. 23 of the specification; in Example 4, p. 25, lines 8-12 and Table 4 of the specification; in Example 5, p. 26, lines 6-9 and Table 5; and in Example 6, p. 27, lines 16-24 and Table 6 on p. 29 of the specification. Accordingly, the claimed method of Applicants' invention effectively reduces loss of metal-chelate affinity particles that would otherwise occur during routine manipulations of the particles and, thus, provides a significant increase in yields that may be obtained using metal-chelate affinity particles.

As noted above, the claims of this application are now directed to preferred embodiments of Applicants' method, i.e., wherein the detergent is used in the range of 0.0005% - 2% (v/v) to reduce loss of metal-chelate affinity particles in a method for isolating a fusion protein comprising a peptide, polypeptide, or protein fused to an affinity peptide tag consisting of a plurality histidine residues. Particularly preferred embodiments as claimed herein include methods comprising the use of metal-chelate, magnetic affinity particles, the use of a fusion protein comprising an affinity peptide tag consisting of six consecutive histidine residues, and the use of a particular species of metal-chelate affinity particles, i.e., nickel-nitriloacetic acid (NTA) agarose beads.

Nowhere does Zhang's method for detecting targeted nucleic acids provide a teaching or suggestion for reducing metal-chelate affinity particle loss during affinity particle-based procedures for isolating fusion proteins by contacting the metal-chelate affinity particles with a detergent according to Applicants' invention. Similarly, Weisburg's description of a standard lysis buffer containing a detergent in a method of identifying ribosomal RNA and genes (i.e., nucleic acids) from spirochetes fails to recognize the problem of losing metal-chelate affinity particles during routine manipulations of the particles in methods of isolating fusion proteins. Clearly, Weisburg fails to teach each and every element of or even to suggest Applicants' claimed methods. Accordingly, Zhang and Weisburg, each, fail to support a rejection of the claims under 35 USC §§ 102 and 103.

The protocol for using metal-chelate affinity particles in McCoy is devoid of any mention of the technical problem of affinity particle loss during isolation procedures. Since McCoy cannot solve what it does not even recognize as a problem, it does not cure the deficiency in Zhang and cannot provide a suggestion of Applicants' claimed invention to those of ordinary skill in this art. Hence, the combination of Zhang and McCoy fails to make Applicants' claimed invention obvious under 35 USC § 103.

Gallant's description of using the detergent CHAPS in an HPLC protocol for purifying an enzyme or the production of new inhibitors of the enzyme apopain also completely fails to recognize the problem of how to reduce loss of metal-chelate affinity particles in a method for isolating fusion proteins. Accordingly, Gallant in combination with the nucleic acid isolation methods of Zhang does not make Applicants' claimed methods obvious under 35 USC § 103.

Stein's description of using cationic wetting agents in a continuous method of separating complex mixtures of fatty alcohols based on differential melting temperatures provides no relevant teaching or suggestion for how a person skilled in this art might modify the methods of isolating target nucleic acids according to Zhang to arrive at Applicants' claimed methods that reduce loss of metal-chelate affinity particles in methods for isolating fusion proteins.

Similarly, Tsaur's description of a polyvinyl alcohol or various hydrophilic polymers that can reversibly cross-link with the hydrophobic polymer core of a composite detergent composition and Taoda's description of an environment purifying material in which particles of titanium oxide (photocatalyst) coated with calcium phosphate to decompose proteins, bacteria, and viruses, fails to provide any relevant suggestion that would transform the teaching of Zhang into a suggestion of Applicants' claimed methods for reducing loss of metal-chelate affinity particles in a method of isolating a fusion protein.

The above comments clearly show that none of the references or combinations thereof cited by the Examiner teaches or suggests Applicants' claimed *methods*. The Examiner urges that the steps of Applicants' methods are suggested by a listing of *compositions*, without disclosure of the problem solved by Applicants' methods. Applicants respectfully submit that the Examiner's view reads too much life into such inert references. A person of ordinary skill in the art would have to consult Applicants' specification to interpret the references and obtain what the Examiner asserts is there. This is hindsight of the sort which the person of ordinary skill in the art prior to the invention is incapable of as a matter of law. Accordingly, reconsideration and withdrawal of the rejections are respectfully requested.

CONCLUSION

Applicants note that none of the references cited by the Examiner, alone or in combination, describes the problem of losing metal-chelate affinity particles in protein purification methods, and none provides a method of solving this technical problem. It cannot be determined whether any reference or combination of references experienced the phenomenon of losing affinity particles as a technical problem recognized by Applicants. The Examiner apparently argues that the references inherently describe Applicants' methods merely because affinity particles or various detergents recited in Applicants' method claims are listed; but such cataloguing of materials teaches no method requiring their use. As Applicants have noted previously, the claimed invention is not a composition of listed materials. Rather, Applicants have claimed a particular *method* to overcome a particular problem that is not even recognized in any of the references relied on by the Examiner to reject the claims.

In view of all of the above comments and the amendments herein, Applicants respectfully submit that the claims, as amended herein, are in proper form for allowance. Accordingly, the Examiner is respectfully requested to enter the amendments, withdraw the rejections, and pass the present application to issue.

Respectfully submitted,

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CERTIFICATE OF MAILING

The undersigned hereby certifies that the items of correspondence referred to above are being deposited with the U.S. Postal Service First Class Mail, postage prepaid, in an envelope addressed to the Commissioner for Patents, Box RCE, Washington, DC 20231 on the date indicated below:

February 10, 2003
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Stephanie L. Leicht
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AMENDED CLAIMS IN U.S. SERIAL NO. 09/812,541
(marked up claims showing deletions and cancellations by ~~strikethrough~~, additions by underlining, and new claims as indicated)

2. (twice amended) A method for isolating a fusion protein, wherein said fusion protein comprises a peptide, polypeptide, or protein and an affinity peptide tag consisting of a plurality of consecutive histidine residues ~~molecule~~ from a sample in a vessel, comprising the steps of:

(a) combining the sample containing the fusion protein ~~a peptide, polypeptide, or protein molecule of interest~~ with metal-chelate affinity particles suitable for binding said fusion protein ~~molecule~~, said metal-chelate affinity particles being insoluble in the sample;

(b) collecting the metal-chelate affinity particles;

(c) separating the metal-chelate affinity particles from the unbound remainder of the sample;

(d) optionally, resuspending the metal-chelate affinity particles in a solution;

(e) optionally, eluting said fusion protein ~~molecule~~ from the metal-chelate affinity particles, followed by separating the metal-chelate affinity particles from said eluted fusion protein ~~molecule~~;

wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of 0.0005% - 2% (v/v) detergent sufficient to reduce loss of metal-chelate affinity particles during any separation or collection step, in comparison to the same method performed in the absence of detergent.

3. (amended) The method according to Claim 2, wherein the combining step (a) is carried out in the absence of detergent, but detergent is added prior to the collecting ~~separation~~ step (b).

~~5. The method according to Claim 2, wherein said molecule is a fusion protein or peptide.~~

~~6. The method according to Claim 5, wherein said fusion protein is a protein or peptide fused to a metal chelating group.~~

~~7. The method according to Claim 6, wherein said metal chelating group is two or more histidine residues.~~

8. (amended) The method according to Claim 2 ~~6~~, wherein said affinity peptide tag ~~metal chelating group~~ is six consecutive histidine residues.

13. (amended) The method according to Claim 2, wherein said metal-chelate affinity particles are selected from the group consisting of ferromagnetic beads, superparamagnetic beads, and combinations thereof.

14. (twice amended) The method according to Claim 2, wherein said metal-chelate affinity particles are composed of materials selected from the group consisting of agarose, silica, nitrocellulose, cellulose, acrylamide, latex, polystyrene, polyacrylate, polymethacrylate, polyethylene polymers, glass particles, silicates, metal oxides, apatites, and combinations thereof.

~~15. The method according to Claim 14, wherein said particles are coated with an affinity ligand selected from the group consisting of antibodies for a particular antigen, antigens for a particular antibody, antibodies recognizing a class of molecules, streptavidin, streptavidin-tagged fusion proteins, biotin, biotin-tagged fusion proteins, glutathione, cellulose, amylose, ion exchange groups, hydrophobic interaction groups, binding molecules for cell-surface markers, phage ligands, antibodies recognizing cell or phage surface antigens, and polypeptides, nucleotides or small molecules capable of affinity interactions with a binding partner selected from the group consisting of peptides, polypeptides, and proteins.~~

~~16. The method according to Claim 2, wherein said detergent, where present, is at a concentration of from about 0.0005% to 2.0% (v/v).~~

17. (amended) The method according to Claim 2 ~~16~~, wherein said detergent is selected from a group consisting of nonionic detergents, anionic detergents, zwitterionic detergents, cationic detergents, and combinations thereof.

19. (twice amended) The method according to Claim 17, wherein said nonionic detergent is polyoxyethylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).

~~32. The method according to Claim 2, wherein the detergent is polyoxyethylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).~~

34. (twice amended) A method for isolating a fusion protein, wherein said fusion protein comprises a peptide, polypeptide, or protein molecule and an affinity peptide tag consisting of a plurality of consecutive histidine residues from a sample in a vessel, comprising the steps of:

(a) providing a multiplicity of metal-chelate affinity particles and incubating said metal chelate affinity particles in the presence of a detergent;

(b) combining the sample containing the fusion protein ~~a peptide, polypeptide, or protein molecule of interest~~ with metal-chelate affinity particles suitable for binding said fusion protein ~~molecule~~, said metal-chelate affinity particles being insoluble in the sample;

(c) collecting the metal-chelate affinity particles;

(d) separating the metal-chelate affinity particles from the unbound remainder of the sample;

(e) optionally, resuspending the metal-chelate affinity particles in a solution;

(f) optionally, eluting said fusion protein ~~molecule~~ from the metal-chelate affinity particles, followed by separating the metal-chelate affinity particles from said eluted fusion protein ~~molecule~~;

wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of 0.0005% - 2% (v/v) detergent, wherein the use of detergent is sufficient to reduce loss of metal-chelate affinity particles during any separation or collection step, in comparison to the same method performed in the absence of detergent.

~~36. The method according to Claim 34, wherein said molecule is a fusion protein or peptide.~~

~~37. The method according to Claim 36, wherein said fusion protein is a protein or peptide fused to a metal-chelating group.~~

~~38. The method according to Claim 37, wherein said metal-chelating group is two or more histidine residues.~~

39. (amended) The method according to Claim 34 ~~37~~, wherein said affinity peptide tag ~~metal-chelating group~~ is six consecutive histidine residues.

44. (twice amended) The method according to Claim 34, wherein said metal-chelate affinity particles are selected from the group consisting of ferromagnetic beads, superparamagnetic beads, and combinations thereof.

45. (twice amended) The method according to Claim 34, wherein said metal-chelate affinity particles are composed of materials selected from the group consisting of agarose, silica, nitrocellulose, cellulose, acrylamide, latex, polystyrene, polyacrylate, polymethacrylate, polyethylene polymers, glass particles, silicates, metal oxides, apatites, and combinations thereof.

~~46. The method according to Claim 45, wherein said particles are coated with an affinity ligand selected from the group consisting of antibodies for a particular antigen, antigens for a particular antibody, antibodies recognizing a class of molecules, streptavidin, streptavidin-tagged fusion proteins, biotin, biotin-tagged fusion proteins, glutathione, cellulose, amylose, ion exchange groups, hydrophobic interaction groups, binding molecules for cell surface markers, phage ligands, antibodies recognizing cell or phage surface antigens, and polypeptides capable of affinity interactions with a binding partner selected from the group consisting of peptides, polypeptides, and proteins.~~

~~47. The method according to Claim 34, wherein said detergent, where present, is at a concentration of from about 0.0005% to 2.0% (v/v).~~

48. (amended) The method according to Claim 34 ~~47~~, wherein said detergent is selected from a group consisting of nonionic detergents, anionic detergents, zwitterionic detergents, cationic detergents, and combinations thereof.

64. (twice amended) A method for isolating a fusion protein, wherein said fusion protein comprises a peptide, polypeptide, or protein molecule and an affinity peptide tag consisting of a plurality of consecutive histidine residues from a sample in a vessel, comprising the steps of:

(a) combining the sample containing the fusion protein ~~a peptide, polypeptide, or protein molecule of interest~~ with metal-chelate, magnetic affinity particles suitable for binding said fusion protein ~~molecule~~, said metal-chelate, magnetic affinity particles being insoluble in the sample;

(b) applying a magnetic field to the vessel so as to attract and immobilize the metal-chelate, magnetic affinity particles;

(c) separating the unimmobilized remainder of the sample from the immobilized metal-chelate, magnetic affinity particles;

(d) optionally, resuspending the metal-chelate, magnetic affinity particles in a solution;

(e) optionally, eluting said fusion protein ~~molecule~~ from the metal-chelate, magnetic affinity

particles, followed by separating the metal-chelate, magnetic affinity particles from said eluted fusion protein ~~molecule~~;

wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of 0.0005% - 2% (v/v) detergent sufficient to reduce loss of metal-chelate, magnetic affinity particles during any separation or collection step, in comparison to the same method performed in the absence of detergent.

66. (twice amended) A method for isolating a fusion protein comprising a peptide, polypeptide, or protein and an affinity peptide tag consisting of a plurality of consecutive histidine residues ~~molecule~~ from a sample in a vessel, comprising the steps of:

(a) providing a multiplicity of metal-chelate, magnetic affinity particles and incubating said metal-chelate, magnetic affinity particles in the presence of a detergent;

(b) combining the sample containing the fusion protein ~~a peptide, polypeptide, or protein molecule of interest~~ with said metal-chelate, magnetic affinity particles suitable for binding said fusion protein ~~molecule~~, said metal-chelate, magnetic affinity particles being insoluble in the sample;

(c) immobilizing the metal-chelate, magnetic affinity particles by applying a magnet to said vessel;

(d) separating the remainder of the sample from the immobilized metal-chelate, magnetic affinity particles;

(e) optionally, resuspending the metal-chelate, magnetic affinity particles in a solution;

(f) optionally, eluting said fusion protein ~~molecule~~ from the metal-chelate, magnetic affinity particles, followed by separating the metal-chelate, magnetic affinity particles from said eluted fusion protein ~~molecule~~;

wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of 0.0005% - 2% (v/v) detergent, wherein the use of detergent is sufficient to reduce loss of metal-chelate, magnetic, affinity particles during any separation or collection step, in comparison to the same method performed in the absence of detergent.

70. (new) The method for isolating a fusion protein according to any one of Claims 64-66, wherein said fusion protein comprises a peptide, polypeptide, or protein and an affinity peptide tag consisting of six consecutive histidine residues and said metal-chelate, magnetic affinity particles are nickel-nitrilotriacetic acid agarose beads.